Investigating the Nitrogen Fertilization Potential of Human Hair

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ABSTRACT

The widespread use of synthetic nitrogen fertilizers in agriculture poses significant environmental and health risks including groundwater contamination and greenhouse gas emissions. This study investigates the potential of human hair, an organic material with a high nitrogen content of 15%, as a sustainable alternative to synthetic nitrogen fertilizers. An incubation experiment was conducted to evaluate the decomposition of human hair in low-nutrient California grassland soils and its impact on soil nitrogen availability and greenhouse gas emissions. Results indicate that human hair amendments can act as a slow-release nitrogen fertilizer, increasing plant-available nitrogen in soils over time. Microbial shifts revealed the involvement of keratinophilic fungi in breaking down hair and the potential importance of soil characteristics and fungal availability to hair decomposition. The calculated global warming potential from the hair-treated incubated soils was larger than that of typical cropping systems, mainly attributed to the large N_2O emissions in the incubation experiment and the lack of plants to take up the produced soil inorganic nitrogen. Further research should investigate the decomposition of hair in field settings to evaluate its impact on crop yield and climate change. The large-scale use of hair waste in agriculture could provide a sustainable and regenerative alternative to synthetic fertilizers.

KEYWORDS

Human Hair, Decomposition, Keratinophilic Fungi, Agriculture, Nitrogen Fertilizer

INTRODUCTION

The widespread use of synthetic nitrogen (N) fertilizers in California agriculture poses dangers to both the environment and human health. Between 1973 and 2005, synthetic N fertilizer application rates increased an average of 25% and it is estimated that 69% of N added annually to cropland is from synthetic fertilizers (Tomich et al. 2016). Nitrogen use efficiency (NUE) measures the ratio of plant N uptake to the amount of N fertilizer applied and in California, practically every high value crop averages a NUE below 50% which means that lots of excess N remains in the soil (Tomich et al. 2016). Although N fertilizers are a necessary input to agriculture, their use has many negative externalities. Soil N is used by microbial organisms that emit nitrous oxide (N_2O), a powerful greenhouse gas (GHG) with a global warming potential 273 times larger than that of CO_2 on a per mole basis over a 100 year time frame (Martínez-Dalmau et al. 2021) (IPCC, 2021). Agriculture is the largest anthropogenic source of N_2O representing 56% of total anthropogenic N_2O emissions from 2010-2019. (Tian et al 2023). Through mineralization and nitrification, microorganisms produce inorganic compounds (NH_4^+ and NO_3^-) that can be used by plants as nutrients. However with low NUE, NO_3^- can leach into groundwater and integrate into local watersheds causing water quality issues and eutrophication (EPA, 2023). The percolation into groundwater can also cause health issues as ingestion of contaminated drinking water is linked to methemoglobinemia (blue baby syndrome), various cancers, adverse reproductive outcomes, diabetes, and thyroid conditions (Ward 2009).

Due to the high soil mobility and availability of inorganic N fertilizer in soils, there has been an effort to produce slow release fertilizers that could retain nutrients in the soil for longer timescales (Duen et al. 2023). This potentially could reduce N accessibility to microorganisms, mitigate N_2O emissions and allow time for the crop roots to develop and absorb that N. However, inorganic fertilizers that are slow release are more expensive, making their accessibility limited for growers (Chien et al. 2009). A better alternative is sustainable agricultural management strategies including the application of soil amendments that have the potential to reduce N_2O emissions. For example, applying nutrient rich compost lowers the need for synthetic fertilizers and reduces N_2O emissions because it releases nutrients to plants slowly limiting the buildup of excess soil N (Hatano 2021). Compost addition can also sequester carbon, improve water retention, and improve aeration (Sayare et al. 2020). Similarly, biochar, a soil amendment produced through

pyrolysis of biomass from agriculture and forestry wastes can significantly decrease N_2O emissions, increase soil N availability and retention, improve soil fertility, improve crop productivity, and sequester carbon when applied to soils (Cayuela et al. 2013) (Liao et al. 2020). Apart from amending soils, conservation tillage practices are an area of sustainable agriculture that can positively influence N_2O emissions as well. Conservation tillage practices aim to preserve soil structure while reducing nutrient runoff and GHG emissions. Generally, conservation tillage practices such as reduced tillage or no tillage lead to reduced N_2O emissions when compared to conventional tillage (Wang et al. 2021), however some studies suggest that conservation tillage practices actually increase N_2O emissions (Mei et al. 2018). The discrepancies in this trend are likely due to variance in factors such as soil type and crop rotation (Hassan et al. 2022).

Human hair, a high-N content material (~16%), could also potentially be used as an alternative to synthetic N fertilizers (Gupta 2014). Research into the use of human hair as a soil amendment is limited, but a controlled container experiment where hair was applied to soils of crop plants suggests that the addition of hair to soil increases ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, N uptake, and crop yield (Zheljazkov 2005). Research also demonstrated that moisture and the presence of keratinophilic fungi helps decompose hair; however, in an ambient atmosphere hair decomposition is slow (Sharma & Sharma 2010). There is some evidence of agricultural hair use around the world, both directly such as in some Indian communities (Gupta 2014), and as a compost, mixed with manure in traditional Chinese agriculture (Liu et al. 2014).

Valued at USD 6.46 billion in 2022 (Grand View Research 2023), the global human hair trade is a large and growing industry focused on the use of human hair for the production of products like wigs and extensions. From 1991 to 2020, the total trade value increased 23-fold (Wang et al. 2024). However, the human hair trade still represents only 1-2% of the total mass of hair produced globally, with most human hair waste ending up in landfills (Gupta 2014). In landfills, hair decomposes anaerobically producing methane (CH_4), a potent GHGs with a global warming potential 28.9 times larger than that of CO_2 on a per mole basis over a 100 year time frame (Zheljazkov 2005) (IPCC, 2021). Advancements in alternative uses for hair could reduce the impact of waste on the environment by promoting a sustainable life-cycle of a regenerative material.

This study aimed to evaluate the effect of human hair applied on low nutrient California grassland soils. The goals were to assess the potential effectiveness of human hair as a slow-release

N fertilizer in soils and determine potential GHG emissions. In a controlled incubation experiment, human hair was applied to soil to examine how it breaks down over time. The inorganic N levels of the soil were measured and compared between treatment and control plots to determine hair-derived plant-available nutrients to the soil. Soil pH was measured to assess the effects of hair on soil acidity. Greenhouse gas concentrations were collected to measure gas flux and determine the impact of hair on emissions when applied to soil.

METHODS

Site description

The study site is located in a coastal grassland in Nicasio, California (38.068 N, 122.718 W). Annual precipitation averaged 950 mm/yr (38-year mean). Soils were derived from Franciscan me´lange and classified as mollisols in the Tocaloma-Saurin-Bonnydoon series (Ryals and Silver 2013). This area had a mixed landscape with woodland oak forest and rangeland with cattle grazing. Plant growth of annual grasses occurred during the rainy season from October to May and the vegetation dries out during the dry season. Soils had a mean percentage of clay, silt, and sand of 16%, 41%, and 43% (Ryals et al. 2014). Soils were collected from 0-10 cm depth during the dry season of 2023. These soils were not irrigated or tilled and were typically low in mineral N concentrations.

Soil collection

The purpose of using low N soils was to try and ensure that during the experiment any enhancement in microbial activity was a result of the soil amendments and not lasting changes from overly active soil. Using a soil core, I collected several random 1-10 cm depth samples from within a plot at the ranch, totalling over 34 kilograms of material. In the lab, I combined the soil samples in a large plastic tub, breaking up large aggregates and mixing the soil by hand until it was visually homogeneous. I also handpicked rocks and larger pieces of grass and roots from the soil.

Methodology

Human hair pre-treatment and carbon (C) and N characterization

A total of 203 g of dye-free human hair was donated from a local hair salon. I cut it into small pieces (less than a cm long) and placed it on an aluminum foil sheet to avoid contamination and static electricity effects on the walls of the plastic container. I homogenized the soil by hand for approximately 20 minutes. Because there was no published method on how to grind hair to determine the hair C and N content via elemental analysis, I attempted several methods (SPEX Mill, coffee grinder and freeze-dry). The freeze-dry method was successful and consisted on placing a hair subsample in a large mortar, adding liquid N, freeze-drying the hair and grinding it manually with a mortar and pestle. I ground nine subsamples collected across the homogenized hair sheet.

For elemental analyses, I weighed the 9 hair subsamples using a microbalance (Mettler Toledo XS3DU), rolled into small aluminum tin cups, inserted in the sample carousel of the elemental analyzer (Thermo Scientific Flash 2000 CHNS/O Analyzers) for C and N analysis. I made a calibration curve with Atropine as a standard along with the samples to determine the concentration of C and N. I weighed different sample masses to determine the proper sample size for the elemental analysis. One mg of hair produced a signal that was in the mid range concentration value for C and N found in the EA performed calibration curve. The C and N values obtained were 48.2 ± 0.672 % and 14.6 ± 0.286 %, respectively. The measured C and N concentrations were within the known hair C and N range (Gupta 2014). Based on the hair C and N results I determined the amount of hair needed to apply to the soils as a fertilizer (see below).

Experimental design

We designed a soil incubation experiment with replicates of soils under control conditions and amended with hair using an equivalent amount of N to that applied to agricultural land. To determine the amount of hair to add to soil, I used the high end of the range of California crops' N fertilization application rates (145.71 to 184.94 kgN/ha) (Rosenstock 2013). With the use of soil bulk density for the collected soils (1.31g cm^{-3}, Yocelyn Villa, personal communication), and using

unit conversion factors to determine the amount of hair needed to be applied to 100 g of soil (see Equation 1), I obtained a value of 0.0975g of hair. However, with the goal of detecting any potential correlation between the hair treatment and the dependent variables, I doubled the amount and applied 0.2 grams of hair to each jar.

Equation 1:

$$\frac{(184.9\ kgN\ \times\ 1\ ha\ \times\ 1\ m^2 \times\ 1\ cm^3 \times\ 1000\ gN\ \times\ 100\ g\ hair\ \times\ 100\ g\ soil)}{(1\ ha\ x\ 100^2\ m^2 \times\ 100^2\ cm^2 \times\ 1.31\ g\ soil\ \times\ 10\ cm\ \times\ 1\ kgN\ \times\ 14.48\ gN)} = 0.0975g\ hair$$

I proposed an incubation time of 8 weeks, based on previous incubation studies run in the Silver lab (Chari et al 2021). However, as the incubation time progressed, variations in N_2O fluxes between control and hair-incubated jars indicated the need to extend the experiment. The final incubation time was 18 weeks (130 days).

Soil sampling

To measure soil physicochemical variables (inorganic N, pH and soil moisture) during the course of the incubation experiment, I conducted weekly destructive soil sampling from triplicate control and hair-amended soils incubated in jars that were designated only for this purpose. Because the initial incubation time was 8 weeks, a total of 48 jars were prepared (24 control and 24 hair amended soils). For the weeks after week 8, we extracted 15 g of soils approximately every 10 days from the jars devoted for gas sampling.

For gas sampling collection, I created five-replicate control and hair-amended soils in jars with a septa in the lid to minimize leakage from the jar and facilitate syringe gas sampling (Figure 1). These jars were used through the entirety of the experiment. I added deionized water to the soils to bring them to field capacity and weighed the jar mass to correct for moisture shifts during the experiment by adding water to the initial jar+soil weight. Moisture correction was made to the destructive and gas sampling jars.

On the same day of each week for the remainder of the project, I added water to the gas sampling jars and brought their weight to that at the start of the experiment. I exposed all jars to outside air by opening them every day (except weekends) for the first 84 days of the experiment to minimize developing anaerobic conditions and then weekly to the end of the experiment as soil

respiration declined indicating that oxygen consumption would also decline over time due to microbial C limitation.

Figure 1: Gas sampling jars.

Gas Sampling

To collect gas samples, I punched a small hole in each jar lid and inserted a rubber septa tight enough to minimize gas leakage when the jars were closed. After the incubation started, I inserted a needle connected to a 30 ml syringe provided with a stopcock valve on the tip. I flushed the jar air three times to homogenize the gas sample inside the jar and then extracted 25 ml of jar air into the body of the syringe. I closed the stopcock valve, pulled the needle out of the septa, and injected the gas sample in an evacuated vial provided with a septa. The gas sample was stored at room temperature and analyzed later by gas chromatography (Shimadzu 14A gas chromatograph).

Gas sampling frequency varied during the duration of the incubation: during the first week it was done daily, and because there were not significant differences in the GHG concentrations between control and hair-amended jars, I reduced the sampling frequency to three days a week (Monday, Wednesday, and Friday), which I maintained for the next 81 days of the experiment except for day 58 and day 60, a Wednesday and Friday that were missed due to a holiday. From day 84 to the end of the incubation I collected samples weekly. I input collected gas samples into the gas chromatograph which returns the peak areas of CO_2, CH_4, and N_2O. The peak area values represent the amount of each compound detected. We calculated the samples' GHG concentrations by means of a calibration curve using a GHG in gas standard tanks (Scott Marrin), with the following concentrations: 1008 ppm, 10 ppm and 9.9 ppm for CO_2, CH_4 and N_2O, respectively.

Before calculating GHG fluxes, I scaled the GHG concentration values obtained after week 8, to the original mass (100 g of wet soil) to account for the change in soil mass as I began removing soil from the gas jars. Because after week 8 I removed 15 g of wet soils every week, I multiplied the GHG concentrations by the ratio of initial wet soil/(current wet soil- removed soil). Once all GHG concentration values were referenced to the total soil wet mass (100 g soil), I calculated GHG fluxes using the change in the concentration of GHGs at time zero (at the moment of jar closure) and the jar concentration at the next time step (that varied depending of the gas sampling frequency, see below) (Equation 2)

Equation 2:

$$\frac{(GHG\ ppm_T - GHG\ ppm_{T0})}{(T - (T-1))} = ppm/Day$$

I converted gas flux from ppm/day to atm/day and then used the ideal gas law to convert to grams/day. (Equation 3)

Equation 3:

$$\frac{(atm/day \times molar\ mass\ (g/m) \times jar\ volume(L))}{(0.082\ \frac{L \cdot atm}{mol \cdot K} \times 298\ K)} = grams/day$$

Gravimetric water content

While conducting the weekly destructive sampling, soils were analyzed for gravimetric water content by drying approximately 10 g of soil to a constant weight at 105ºC (Brady and Weil 2008). This allowed the changes in soil water content to be tracked over time. As the project was extended past the first eight weeks, I stopped measuring gravimetric water content.

Inorganic nitrogen

To measure ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the soil, each week, I measured 15 g (±0.1) from each jar into specimen cups filled with 76 mL of 2 M KCl solution. I shook the 6 cups (3 control, 3 treatment) for 1 hour on an orbital shaker with 2 blanks specimen cups that contained 2 M KCl and were treated similarly to the samples. After being shaken, I poured the samples into funnels lined with KCl soaked coffee filters and filtered the samples into empty specimen cups until all the liquid had transferred. I then froze the filtered samples at -18º C until analysis.

After the course of the incubation, the samples were analyzed using a colorimetric discrete analyzer (Seal Analytical, Inc. Mequon, WI, USA, Model: AQ3000) to determine soil N content. A cadmium reaction using the Griess-ilosvay method was used to determine NO_3^- content. And the indophenol blue method (Mulvaney 1996) was used to determine NH_4^+ content.

pH

During the weekly sampling, pH was measured in a slurry of 5 g of soil in 5 ml of deionized water before being shaken for 1 minute. Ten minutes after the water was initially added, I used a pH meter (Denver Instruments, Bohemia, New York, USA) to measure the slurry pH (Denmead 2008). As the project was extended past the first eight weeks, I stopped measuring pH until the last sampling day, February 1st.

Statistical analysis

All statistical tests were performed in JMP Pro Statistical Discovery software. We ran one way analysis of variance (ANOVA) tests to compare mean GHG fluxes and mean inorganic N concentrations between the treatments. We used the method of least squares to determine if there was significant correlation between time and pH or inorganic N concentrations. To determine correlation between all the collected variables, we used the restricted maximum likelihood method to run a multivariate correlation analysis.Average values are reported with standard error, unless otherwise indicated in the text. Significance was determined as $p < 0.05$.

Global Warming Potential Calculations

We used the trapezoidal rule to integrate the daily average GHG mass production rate (mg day^{-1} for CO_2 and μg day^{-1} for N_2O and CH_4) over time in order to find the average mass of gas produced per gram of dry soil. Using these values, I was able to calculate the global warming potential of CH_4 and N_2O in kilograms of CO_2 equivalent (CO_2e) per hectare. To convert flux measurements to CO_2 equivalents I used the 100-year GWP values of 29.8 CO_2e for CH_4 and 273 CO_2e for N_2O (IPCC, 2021).

Nitrogen lost as N_2O Calculations

To compare the loss of N as N_2O with that of other mineral N fertilizers, I took the total mass of N_2O produced per jar per gram of dry soil and multiplied it by the soils bulk density and by the soil depth (10 cm) to determine the total mass of N_2O per unit area (see equation 3 below). To approximate NUE of the hair treatment I calculated the amount of N applied that was emitted as N_2O using equation 4.

Equation 3:

$$\frac{ugN2O}{g} \times \frac{1.31\ g}{cm^2} \times\ 10cm\ \times \frac{10000\ cm^2}{m^2} \times \frac{10000\ m^2}{ha} \times \frac{0.000001\ g}{ug} \times \frac{0.001\ kgN2O}{g} = \frac{kgN_2O}{ha}$$

Equation 4:

$$\frac{kgN_2O}{ha} \times \frac{28\ kgN_2O}{44gN2O/mole} = \frac{kgN_2O - N}{ha}$$

RESULTS

Temporal variations of GHG production and physicochemical variables

Greenhouse gas production varied over time in both treatments. CO_2 production was highest at the beginning of the experiment, increasing from 10 to 80 mg/day during the first 20 days before progressively decreasing in both treatments for the remainder of the experiment (Figure 2a). On the other hand, CH_4 production had a brief rise in both treatments starting on day 20 and peaking on day 28 at 7 µg/day in the hair treatment and 5 µg/day in the control treatment. Both treatments progressively decreased from there on until the end of the experiment (Figure 2b). Until the eighth week of the incubation N_2O fluxes were low in both treatments, From day 50 onward N_2O production increased in both treatments; however, in the hair treatment the production was four times higher than that found in the control soils (Figure 2c). The highest N_2O production values (from 24 to 42 µg N_2O/day) were found between day 80 to 130 in the hair treatment.

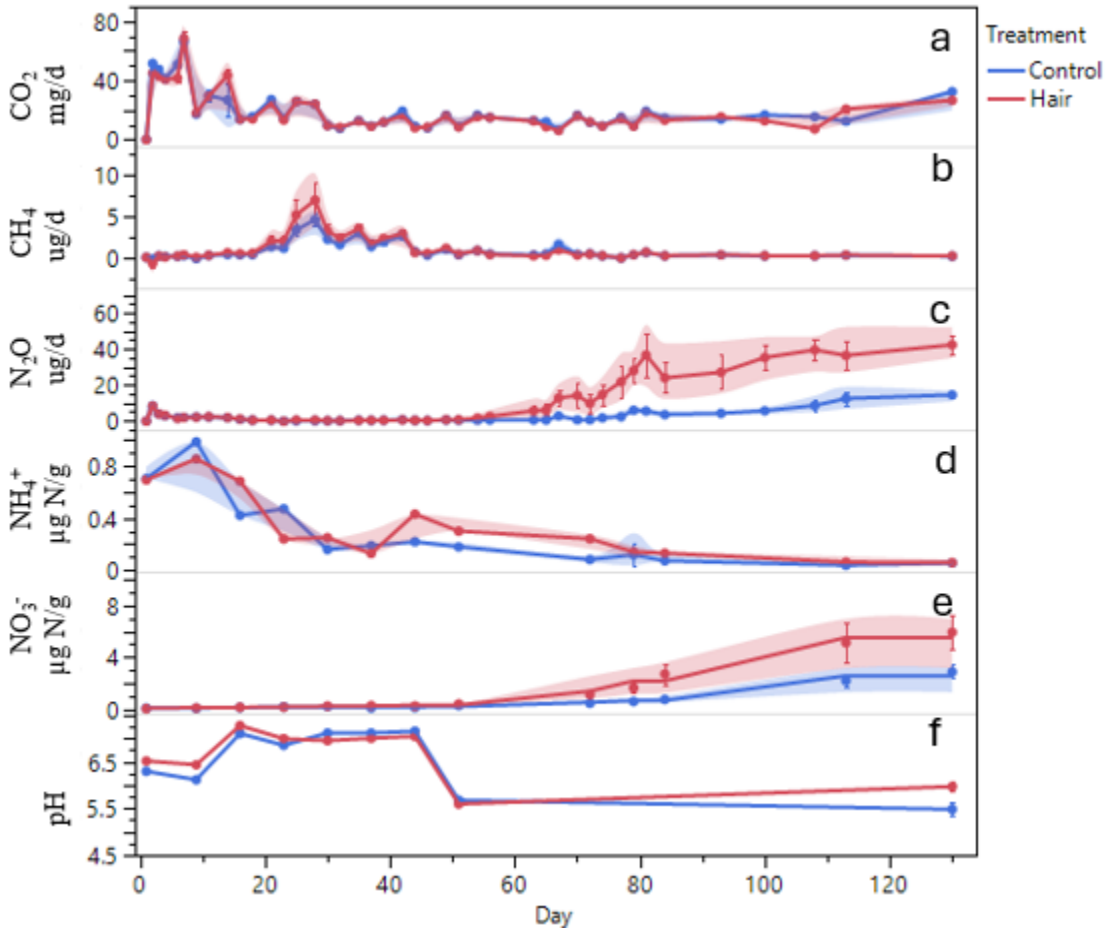

Figure 2: Temporal Variation of mean ± standard error of CO_2 (a), CH_4 (b), N_2O (c) fluxes, NH_4^+ (d) and NO_3^- (e) concentrations, and pH (f) in the control (blue) and hair (red) soil treatments. The shaded area represents the standard deviation confidence interval (99.5).

When comparing mean GHG production over the entire duration of the experiment, I found that N_2O production was significantly larger in the hair treatment (ANOVA, $p < 0.05$). There was no significant difference between the two treatments in CO_2 and CH_4 production (figure 3, Table 1).

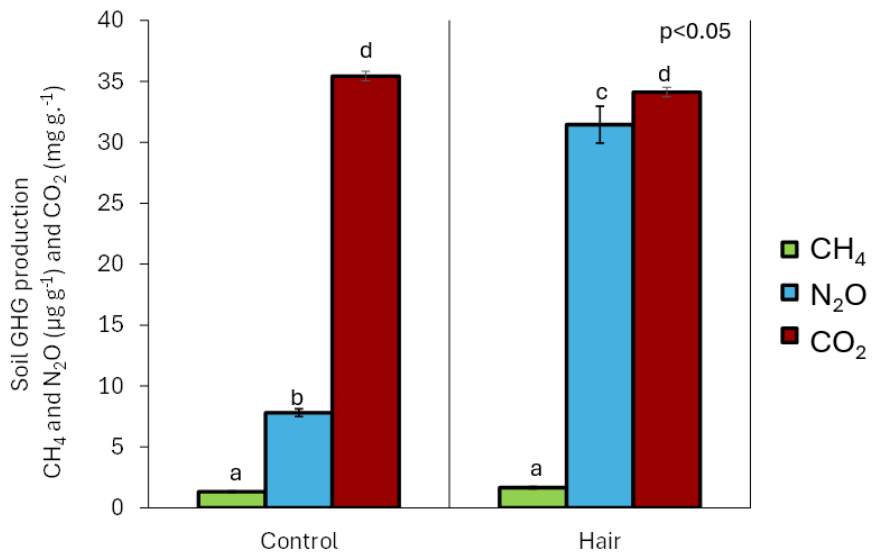

Figure 3. Mean ± standard error of total soil GHG mass production per grams of dry soil over the entire incubation experiment (130 days). The calculation was done by integrating the daily average GHG mass production rate of CO_2 (mg day^{-1}), N_2O and CH_4 (µg day^{-1}) over time using the trapezoidal rule as the integration method (n=130) and dividing it for the soil mass (grams of dry soil). The obtained average GHG production values were statistically compared by Tukey HSD Post-hoc Test ($p<0.05$). Different letters indicate statistically significant differences.

Table 1. Mean ± standard error of total GHG production during the incubation experiment

Treatment	CH_4 (µg g^{-1})	N_2O (µg g^{-1})	CO_2 (mg g^{-1})
Control	1.33 ± 0.04	7.81 ± 0.32	35.44 ± 0.38
Hair	1.67 ± 0.08	31.45 ± 0.39	34.12 ± 0.39

NH_4^+ concentration in the soil was larger during the first 30 days of the experiment for both treatments ranging from 0.16 ± 0.02 to 0.98 ± 0.28 µg N/g (Figure 2d). The control treatment remained low throughout the rest of the experiment with values decreasing to 0.06 ± 0.01 µg N/g (Figure 2d). The hair treatment had another peak, increasing to 0.93 ± 0.5 µg N/g on day 43, then decreasing to 0.14 ± 0.03 µg N/g on day 78, before decreasing more gradually until the end of the experiment. For both treatments NO_3^- concentration remained low until day 43 and then increased over time (Figure 2e). However, for the hair treatment this enhancement was sustained reaching 5.93 ± 1.29 µg N/g by the last day of the experiment whereas for the control treatment was half of

that (2.87 ± 0.5 µg N/g). Soil NO_3^- concentration was an order of magnitude larger than NH_4^+ from day 71 onwards (Figure 2e). Soil pH levels were not significantly different from each other over the course of the experiment. pH ranged from 6.12 ± 0.19 to 7.28 ± 0.01 over the first 43 days before decreasing to 5.6 ± 0.1 on day 50 (Figure 2f). On the last sampling day the control jars were slightly more acidic (5.49) than the hair jars (5.97). When comparing overall mean concentrations for NH_4^+, NO_3^-, and pH per treatment, only NO_3^- was larger in the hair treatment when compared to the control (ANOVA, $p < 0.05$). (0.73 ± 0.14 µg/g control and 1.77 ± 0.36 µg/g hair) There was no significant difference between the two treatments in NH_4^+ concentration and pH level.

The multivariate analysis between GHG production and physicochemical variables show positive correlations between CO_2 production and NH_4^+ and between N_2O production and NO_3^- in both treatments. (REML, $p < 0.05$).

Identification of Keratinophilic fungi

When doing a visual examination of soils, I identified the presence of keratinophilic fungi hyphae around the hair filaments from day 63 onward in all the hair amended soils; however, they were absent in the control soils (figure 4).

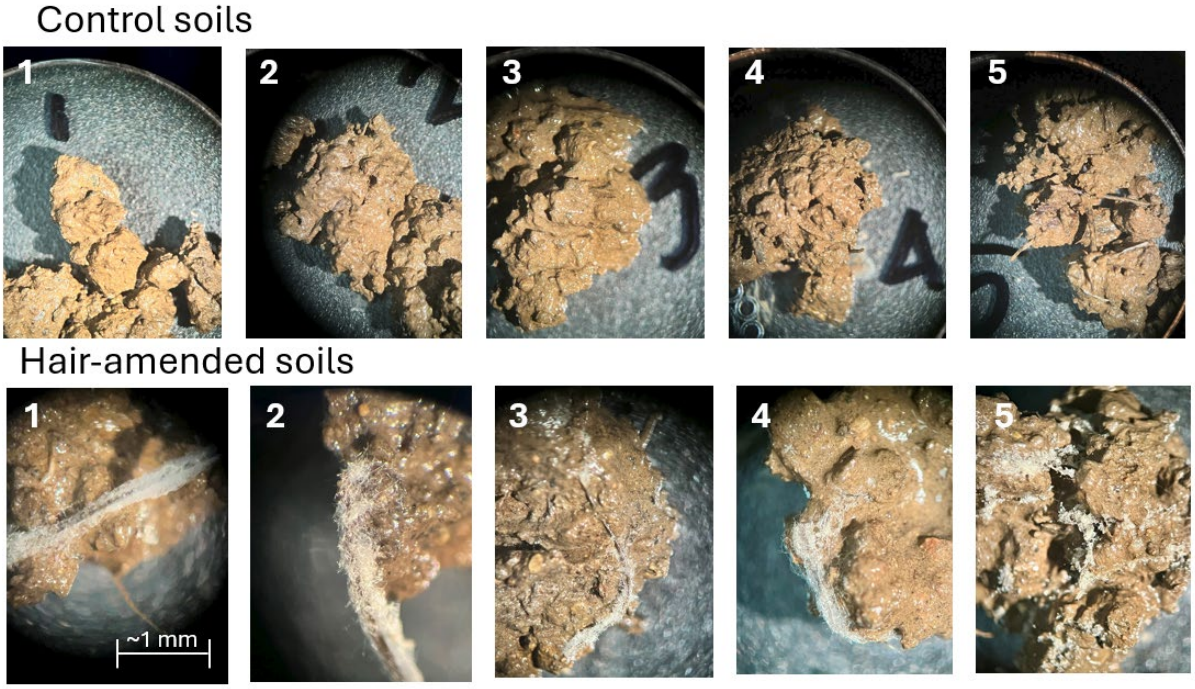

Figure 4: Keratinophilic fungi occurrence from day 63 onward. Gas sampling jars soil subsamples taken from control (top row) and hair-amended treatment (bottom row).

DISCUSSION

These results demonstrate that hair application to soils increases N availability overtime, displaying its ability to successfully perform as a slow release fertilizer. Hair treated soils also had increased N_2O emissions because of the large availability of inorganic N. Hair decomposition began once microorganisms had depleted the organic matter pool in the soils and shifted their activity to breaking down hair. The simultaneous colonization of hair by keratinophilic fungi indicated that it is likely necessary to facilitate hair decomposition. These shifts in microbial activity and their broader implications for agricultural hair use are discussed below.

Soil microbial shifts

The positive correlation between CO_2 and NH_4^+ found in both treatments at the beginning of the experiment indicates the initial decomposition of organic matter. Microorganisms use organic C as an energy source breaking it down into CO_2 (Findlay et al. 2021) and transforming organic nitrogenous compounds to NH_4^+ (Ladd & Jackson 1982). The correlated decline in CO_2 and NH_4^+ shows the decreased organic matter decomposition as microbes ran out of labile organic material to consume, and instead had to shift metabolic activity to breaking down the tougher material, hair.

After the first burst of decomposition in the soils, the early CH_4 peak could be explained by methanogenesis. Methanogenesis is an anaerobic process where methanogenic archaea, or methanogens, ferment products from organic matter decomposition such as CO_2 or acetic acid to produce CH_4 (Buan 2018). Because the CH_4 peak of production came right after the initial decomposition phase, it is likely that carboxylic acid concentrations, derived from organic matter decomposition, were high enough to activate methanogens and enhance CH_4 production via acetate fermentation (Ferry 1992). While the soils from the incubation were not saturated, it is likely that anaerobic microsites could be present to support methanogenesis (Kammann et al. 2009). It is also possible that CH_4 production is the result of a chemical process rather than a microbial one. Hurkcuck et al. (2012) found evidence that there may be an unrecognized chemical process in aerobic soils that produces CH_4 from wet aerobic soils and from the compounds lignin and pectin. However, this is also unlikely because emissions from an abiotic process would likely be more

constant than this temporary peak.

Hair as a source of N for N_2O production

Nitrous oxide can be produced from multiple microbial processes. The most thoroughly understood are nitrification and denitrification. During nitrification, NH_4^+ that is derived from decomposition is oxidized to NO_3^- by nitrifying microorganisms and N_2O can be released. When soil conditions are anaerobic, the NO_3^- that was formed can be used as an electron acceptor for denitrifying microorganisms that reduce it to N_2O, and if the environment is anaerobic enough, further reduce N_2O to N_2 (Firestone and Davidson, 1989).

In our soils, both processes could be occurring. The decline in NH_4^+ and increase in NO_3^- concentrations in both treatments shows the occurrence of nitrification, as the NH_4^+ produced through the initial decomposition of organic material is oxidized to NO_3^-, and contributes to N_2O emissions. It is likely that the soils had anaerobic microsites where denitrification also took place. Large NO_3^- concentrations remained for the duration of the experiment, so the pool of NO_3^- produced from nitrification was likely large enough to support constant denitrification resulting in the increased N_2O emissions that also remained high through the end of the experiment.

Because of the significantly larger average of N_2O production in the hair versus the control treatment (Table 1), we think that NO_3^- concentration and N_2O production is modulated by the keratinophilic fungi. Keratinophilic fungi can colonize various substrates containing the protein keratin, such as feathers, human hair, animal horns, and wool, due to the enzyme keratinase (Kumar et al. 2021). A study by Kumar et al. (2017) found that feather compost with keratinophilic fungi present increased N content, likely by the same mechanisms as in this experiment. One possibility is that the keratinophilic fungi breaks down the hair, freeing NH_4^+ for nitrifying bacteria to consume. Another possibility is that keratinophilic fungi were acting as denitrifiers themselves. There is no evidence of keratinophilic fungi denitrifying but there are other fungi that do so it is a possibility (Maeda et al. 2015).

Potential of hair as a N fertilizer

This experiment reveals that hair amendments in soils can act as a slow-release fertilizer.

Under the conditions of this experiment which were designed to mimic an agricultural setting, it took approximately eight weeks for hair to begin to decompose. This indicates that if applied to crop fields ahead of the time that plants require nutrients, hair could be a compelling alternative to synthetic fertilizers which leach N and release N oxides when not taken up by plants. Composting it with other common compostable materials (food waste, manure, etc) could provide additional N to compost and improve nutrient bioavailability at shorter timescales than when applied unprocessed (Waliczek et al 2023). It is possible that hair in soils needs keratinophilic fungi to decompose successfully and that the amount of time for fungal colonization to start depends on soil types and conditions. A study into the patterns of keratinophilic fungi occurrence found the lowest frequencies in sandy soil and the highest frequencies in chernozem, a highly fertile mollisol (Ren et al. 2023) Additionally, the same study found that keratinophilic fungi occurrence had a positive correlation with pH, silt content, and clay content but a negative correlation with sand content. These results imply that keratinophilic fungi growth is magnified in soils that have increased sorption and water-holding capacity, and thus, soils that are likely richer in nutrients. Correlation of keratinophilic fungi occurrence in rich soils suggests that it is worthwhile to further explore hair's use as an amendment to agriculture since rich soils are used and consequently, keratinophilic fungi are likely present.

Climate benefits over inorganic fertilizer

We calculated the integrated total GHG mass production over time of CH_4 and N_2O expressed as kg CO_2e per gram of dry soil using the global warming potential of each gas (29.8 kg CO_2e/kg CH_4 and 273 kg CO_2e/kg N_2O) in a time frame of 100 years (IPCC, 2021). Control treatments produced 50.3 kg CO_2e/ha of CH_4 and 2793 kg CO_2e/ha of N_2O. Hair treatments produced 63.32 kg CO_2e/ha of CH_4 and 11247 kg CO_2e/ha of N_2O.

When compared to crop systems in terms of global warming potential of CH_4 and N_2O emissions, the hair treatments had much higher values (Figure 5). This is likely because of the lack of plants in the incubation experiment. In the crop system examples, plants take up some of the soil N, in contrast, in the incubation experiment any soil N provided by hair is available for microorganisms to transform to N_2O. Similarly, when calculating the N_2O emission factor (as a percentage of applied N fertilizer), the hair treatment was much higher than crop systems (Figure

6). This is also likely due to the fact that other systems have plants taking in soil N.

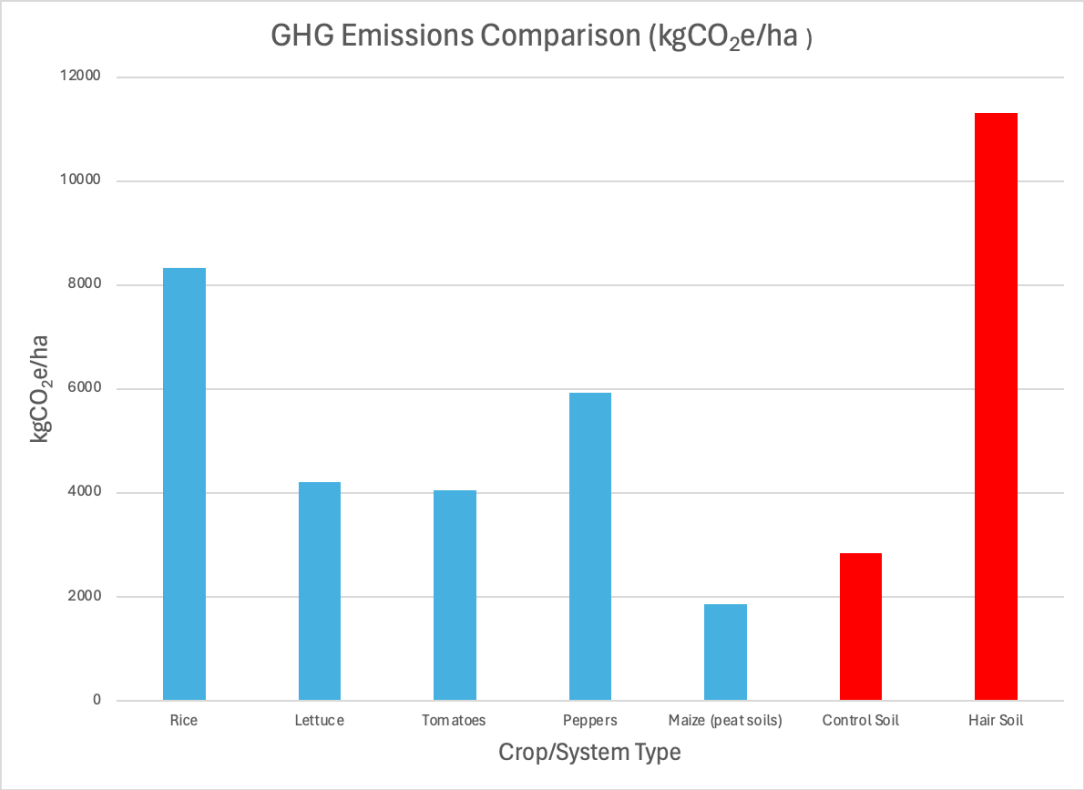

Figure 5: GHG global warming potential comparison. Control and hair treated soils (red bars) GHG global warming potential (for CH_4 and N_2O) expressed as $kgCO_2e\ ha^{-1}$. For comparison, other agricultural crop warming potential values are included (blue bars).

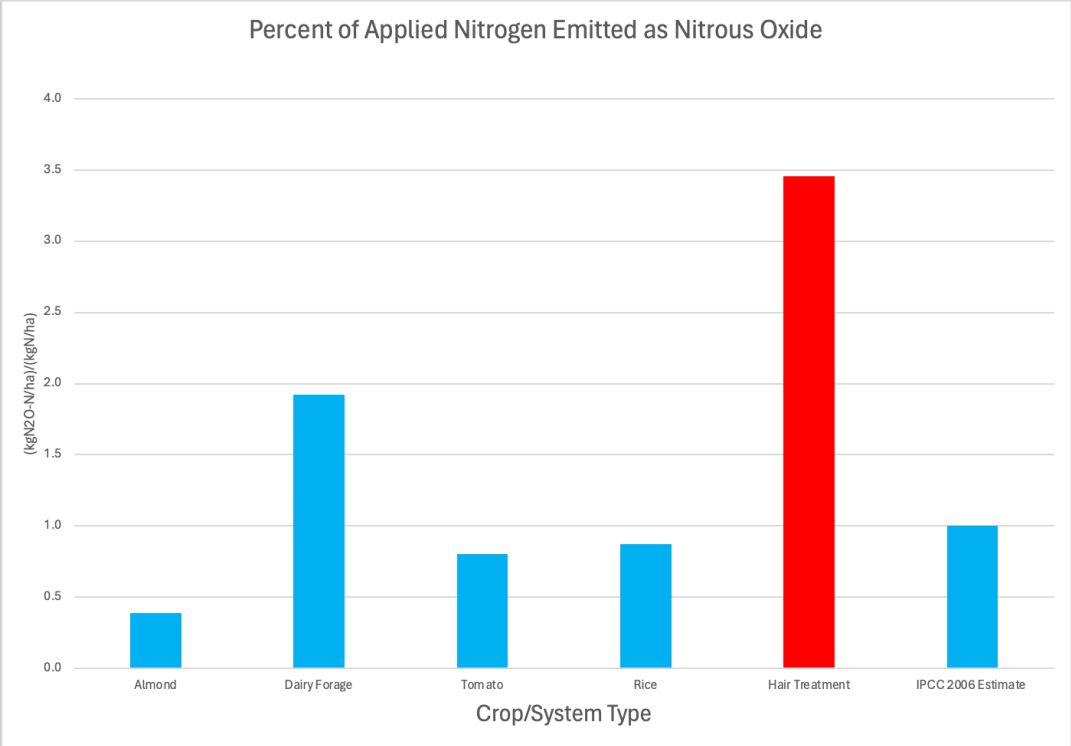

Figure 6: N emitted as N_2O comparison. Hair treated soils (red bar) N_2O emission factors. For comparison, other agricultural crop emission factor values are included (blue bars).

Management Implications

Hair can successfully decompose and dramatically increase the plant available N in agricultural soil. As a regenerative material, the large-scale use of hair could offset the use of harmful synthetic N fertilizers. In California, synthetic fertilizers add 466,293 metric tons of N to the state each year (Tomich et al. 2016). An impact report by Green Circle Salons found that every day, 28,575.3 kg of hair waste is produced by salons in the United States and Canada which would equate to approximately 1512 metric tons of N annually. While this is only a small amount of the N currently used, it is important to consider hair's increased efficiency due to its slow-release of nutrients versus synthetic fertilizer systems that lose roughly half of the N applied to the environment (Tomich et al. 2016). Also worth consideration is the amount of non- human keratin waste that could also be used such as pet hair, fur, feathers, and wool. If hair waste was collected and utilized for agricultural fertilization at an industrial scale, it could be a low-cost and sustainable means of preventing a significant amount of N pollution and GHG emissions while providing N to crops.

Conclusions and Future Directions

This experiment contributed valuable insights into how hair may decompose in agricultural soils and identified areas for future research to investigate. Performing this experiment under field conditions with different soil types and planted crops would help assess the effectiveness of hair as a N amendment to agricultural soils, how it varies with soil types and what is the effect on crop yields. Furthermore, the soils used for this incubation were not highly productive so further research is needed to assess how variables like organic matter content would affect the decomposition of hair. We postulate that more labile organic matter could lengthen the time it takes for hair to break down.

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